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Antiamnesic activity of *Solanum melongena* L. extract

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SUMMARY

Solanum melongena L. (egg plant) is proved to contain antioxidant and neuroprotective agents. So we evaluated the antiamnesic activity of the present extract.

Antiamnesic activity was evaluated using scopolamine, an inducing agent and memory disruptor and employed various behavioral and biochemical parameters like radial arm maze test, active avoidance test. Acetylcholinestarase activity in the brain was measured and oxidative stress was also determined.

Dose dependent reduction in the working and reference memory errors were identified in radial arm maze test. Increased active avoidances were reported after treatment with high dose of extract (400 mg/kg) in active avoidance testing. Acetylcholinestarse levels and oxidative stress parameters were maintained normal in extract treated groups and the values are comparable to standard drug piracetam treatment.

Based on the results of behavioral and biochemical studies, hypothesize that egg plant extract may act directly as a free radical scavenger or regulator to inhibit acetylcholinesterase due to the presence of phytoconstituents mainly flavonoids, polyphenols which might be responsible for exhibiting antiamnesic activity.

KEY WORDS: SOLANUM MELONGENA L. EXTRACT – ANTIAMNESIC ACTIVITY

Background

Memory is the complex process of the brain which involves acquisition of information from the surroundings and consolidation of the acquired information and then retrieving it for future use. Central cholinergic system plays a major role in learning and memory process through various neuronal pathways and neurotransmitters. Deficits occurring in these pathways may result in occurance of various cognitive disorders like amnesia and dementia. Alzheimer's disease (AD) is one of the most common causes of impaired cognitive functions. Besides reduced cholinergic activity, oxidative stress is also one of the major causes for memory loss in AD. Hence, agents which act by reducing oxidative stress and increased cholinergic activity are found to be useful in treating memory impairments (1).

Solanum melongena L. (egg plant) is a plant native of India and is ranked as one of the top ten vegetables in terms of oxygen free radical scavenging capacity (2). Main constituents of egg plant are phenolic compounds, chlorogenic acid and caffeic acids and they were all established as neuroprotective and antioxidant agents (3). In keeping this view in mind the present investigation was carried out on egg plant (*Solanum melongena* L.) to evaluate its antiamnesic activity.

Scopolamine, a muscarinic receptor antagonist, is reported to impair long term potentiation (LTP), and hence it serves as experimental model of AD and thereby used as amnesic agent for evaluation of antiamnesic effect of new drugs (4).

Materials and methods

Collection of Plant Material

The proposed plant material of fresh *Solanum melongena* fruits were collected from Mogilicherla, Warangal district of Andhra Pradesh – India in the month of June. The plant was identified and authenticated by Dr. V. S. Raju, Senior Professor in Department of Botany, Kakatiya University, Warangal, India. The voucher specimen of plant was deposited for further reference.

Preparation of extract

The *Solanum melongena* fruits were first washed well and the seeds were removed from the fruits. The flesh of the fruit was chopped into small pieces (2-4 cm) and shade dried at room temperature. The dried samples were grounded to powder using a grinder. The dried ground powder was passed through a standard 20 mesh size (particle size < 0.850 mm). Shade dried powder was weighed (500 mg) and placed into 15 ml plastic tubes and 10 ml of 80% methanol

was added to it. The mixture was vigorously shaken using a vortex mixer for 2 min, then left in a rotary shaker overnight at ambient temperature to ensure effective extraction. The samples were then centrifuged at 5000 rpm for 15 min and the supernatant was filtered using Whatman filter paper. The residues were then re-extracted two more times with additional 10 ml 80% methanol. All three extracts were combined and concentrated using a rotavapor at reduced temperature and pressure in order to remove the solvent completely. It was dried and kept in a desiccator till experimentation (5).

Animals

All experiments were conducted using Albino Wistar rats (150-200 g) of both the sexes at about 6-8 weeks of age. All animals were procured from Sanzyme Ltd., Hyderabad. The animals were maintained with free access to food and water and kept at 25 ±2°C under a controlled 12 h light/dark cycle. The mice were allowed to acclimatize to the laboratory environment for a week before the start of the experiment. The care and maintenance of the animals were carried out as per the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. The research protocols were approved by the Institutional Animal Ethical Committee (IAEC). The approval number is 13/SPIPS/IAEC/12.

Drugs and chemicals

Scopolamine hydrobromide purchased from Boehringer Ingelheim India, acetylcholine chloride, 5,5-dithio-bis2–nitrobenzoic acid, (Ellman's reagent), acetyl thiocholine iodide, trichloroacetic acid, thiobarbituric acid (TBA) were purchased from Sigma-Aldrich India, piracetam was purchased from Glaxo Smith Kline India.

Acute toxicity study

The acute toxicity was performed according to the OECD 423 guidelines. The extract at the dose of 5, 50, 300 and 2000 mg/kg body weight, was administered to the rats and they were subsequently observed closely for the first 4 h for any untoward symptoms such as tremors, convulsions, exophthalmus, salivation, diarrhea and lethargy followed by observation for a further 14 days. At the end of the experimental period, the animals were observed for any changes in behavioral pattern and mortality. No toxicity was found in all the 4 doses, so the extract was declared safe up to 2000 mg/kg.

Phytochemical studies

The different successive extracts so obtained were subjected to preliminary phytochemical screening by applying different qualitative testes for phytoconstituents. The extract of *Solanum melongena* contains alkaloids, carbohydrates, phenolics, flavonoids, tannins, steroids and saponin glycosides. The presence of these phytoconstituents was confirmed by TLC.

Grouping and treatment protocol

Five groups of animals were made, each group consisting of six rats. The following were the groups.

Group 1: Vehicle control; rats received only vehicle.
Group 2: Positive control (PC); rats received only vehicle against scopolamine (1 mg/kg, i.p.) – induced amnesia.
Group 3: Standard drug (STD) piracetam (200 mg/kg, i.p.) treated rats against scopolamine induced amnesia.
Group 4: Extract of *Solanum melongena* (Test 1) (200 mg/kg, p.o) treated rats against scopolamine induced amnesia.
Group 5: Extract of *Solanum melongena* (Test 2) (400 mg/kg, p.o) treated rats against scopolamine induced amnesia.

Behavioral models

Active avoidance test

Active avoidance test helps to evaluate the associative learning of the animal. The criterion for improved cognitive activity was taken as significant increase in the avoidance response (6).

Evaluation of antiamnesic activity by radial arm maze model

A radial arm maze is used to evaluate working memory in the animals. Each arm (50 x 12 cm) of the eight-arm radial maze extends from an octagonal shaped central hub of 30 cm diameter. The platform is elevated 40 cm above the floor, small black metal cups (3 cm in diameter and 1 cm deep) are mounted at the end of each arm that serve as receptacles for reinforces food (7).

Estimation of acetylcholinesterase activity

The acetylcholinesterase activity was estimated using Ellman's method (8).

Biochemical estimation of markers of oxidative stress

Biochemical tests were conducted 24 h after last behavioral test. The animals were sacrificed by decapitation. Brains were removed and rinsed with ice-cold isotonic saline. Brains were then homogenized with

ice-cold phosphate buffer (pH 8). The homogenates (10% w/v) were then centrifuged at 10,000 rpm for 15 min and the supernatant so formed was used for the biochemical estimations.

Estimation of superoxide dismutase

Superoxide dismutase activity in the brain was determined using photo oxidation of o-dianisidine sensitized by riboflavin method (9). The change in absorbance was recorded for 4 min at 460 nm using spectrophotometer.

Estimation of lipid peroxidation (LPO)

The extent of lipid peroxidation in the brain was determined quantitatively by performing the method as described by Ohkawaka (10). The amount of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid at 532 nm using spectrophotometer.

Estimation of Catalase activity

Catalase activity was assessed by the method of Beers and Sizer (11) based on the ability of catalase to oxidize hydrogen peroxide. The change in absorbance was recorded for 3 min at 1 min interval at 240 nm using spectrophotometer.

Statistical analysis

All experimental groups were composed by 6 animals. The results were presented as the mean ± SEM. Statistical analysis was done by ANOVA followed by Bonferroni's test. P< 0.05 was considered as statistically significant.

Results

Acute toxicity profile

The rats treated with the extract of *Solanum melongena*, 5-2.000 mg/kg, p.o., exhibited normal behavior. They were alert, with normal grooming, touch response and pain response. There was no sign of passivity, stereotypy and vocalization. Their motor activity and secretory signs were also normal. The animals showed no signs of depression. Alertness, limb tone and grip strength as well as the gait of the animals were normal. The extract of *Solanum melongena* was found to be safe up to a dose 2.000 mg/kg in rats.

Effect of Solanum melongena extract on behavioral models

Active avoidance test

In active avoidance test number of avoidances were significantly ($p < 0.05$) less in scopolamine treated group when compared with the control group (tab. 1 and fig. 1). However treatment with standard drug (piracetam) and extract of *Solanum melongena* (200 and 400 mg/kg, p.o.) seemed to show the protective effect significantly ($p < 0.05$) against scopolamine-induced memory impairment by inhibiting the incidence of less number of avoidances. *Solanum melongena* (400 mg/kg) dose shown more prominent results in increasing the avoidance responses compared to (200 mg/kg) dose.

Working memory errors

In radial arm maze, working memory errors were more in scopolamine treated group when compared to control group and indicates memory impairment. While pretreatment with *Solanum melongena* (200 and 400 mg/kg, p.o.) and piracetam (200 mg/kg, i.p.) there was significant ($p < 0.05$) reduction in working memory errors when compared to positive control

Table 1. Effect of *Solanum melongena* extract on active avoidance paradigm against scopolamine induced amnesia.

Treatment groups (n = 6)	Number of avoidance responses
1. Vehicle control	12.50 ± 0.22
2. Positive control	5.83 ± 0.30*
3. Standard	11.17 ± 0.16#
4. Test 1	8.50 ± 0.22#
5. Test 2	9.67 ± 0.21#

*P < 0.05 compared with normal control group. #p < 0.05 compared with scopolamine treated group. The results were presented as the mean ± SEM. Statistical analysis was done by ANOVA followed by Bonferroni's test. P < 0.05 was considered as statistically significant. Values are presented as mean ± SEM, n = 6.

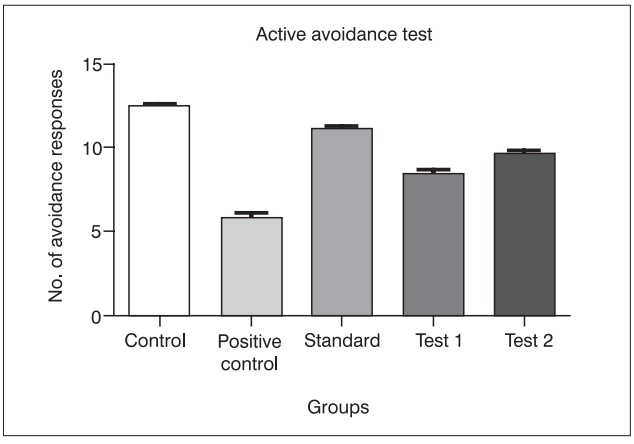

Fig. 1. Effect of *Solanum melongena* extract on active avoidance paradigm against scopolamine induced amnesia.

group as shown in table 2 and figure 2. Higher dose of *Solanum melongena* (400 mg/kg) dose has shown more prominent results in reducing the occurance of working memory errors.

Reference memory errors

The occurrence of reference memory errors were significantly ($p < 0.05$) less in piracetam group and the animal group which received *Solanum melongena* (200 and 400 mg/kg, p.o.) when compared to positive control group as shown in table 3 and figure 3. *Solanum melongena* protected memory by inhibiting the occurrence of more reference memory errors. *Solanum melongena* extract showed dose dependent reduction in occurance of reference memory errors. *Solanum melongena* (400 mg/kg) dose shown more prominent results in reducing the occurance of reference memory errors compared to (200 mg/kg) dose.

Table 2. Effect of *Solanum melongena* extract on working memory against scopolamine induced cognitive impairment.

Treatment groups (n=6)	Working memory errors
Vehicle control	0.6667 ± 0.21
Positive control	1.667 ± 0.21*
Standard	1.000 ± 0.25#
Test 1	1.333 ± 0.21#
Test 2	1.167 ± 0.16#

*P < 0.05 compared with normal control group. #p < 0.05 compared with scopolamine treated group. The results were presented as the mean ± SEM. Statistical analysis was done by ANOVA followed by Bonferroni's test. P<0.05 was considered as statistically significant. Values are presented as mean ± SEM, n = 6.

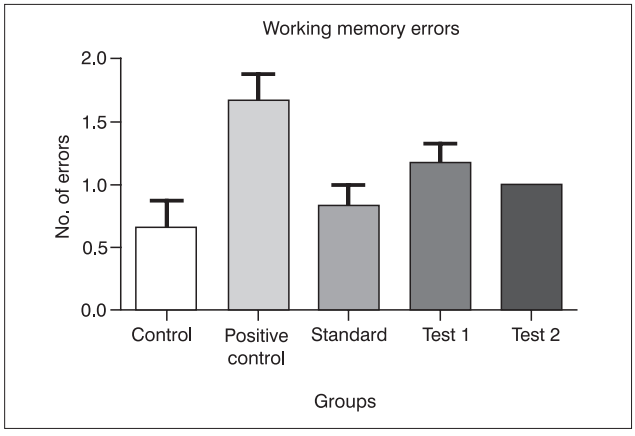

Fig. 2. Effect of *Solanum melongena* extract on working memory against scopolamine induced cognitive impairment.

Table 3. Effect of *Solanum melongena* extract on reference memory against scopolamine induced cognitive impairment.

Treatment groups (n=6)	Reference memory errors
1. Vehicle control	0.6667 ± 0.51
2. Positive control	1.833 ± 0.40*
3. Standard	0.8333 ± 0.75#
4. Test 1	1.167 ± 0.40#
5. Test 2	1.000 ± 0.89#

*P < 0.05 compared with normal control group. #p<0.05 compared with scopolamine treated group. The results were presented as the mean ± SEM. Statistical analysis was done by ANOVA followed by Bonferroni's test. P<0.05 was considered as statistically significant. Values are presented as mean ± SEM, n = 6.

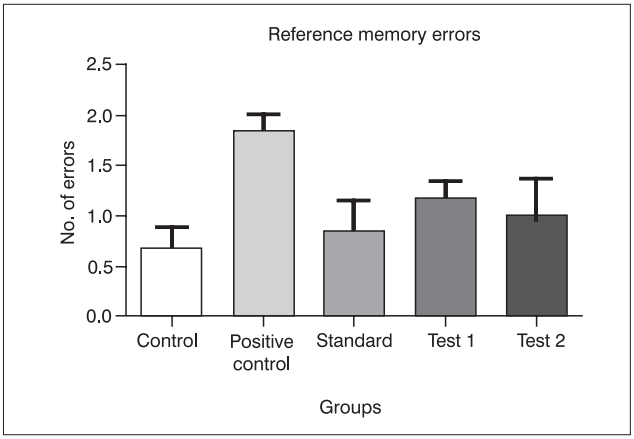

Fig. 3. Effect of *Solanum melongena* extract on reference memory against scopolamine induced cognitive impairment.

Acetylcholinesterase activity

Scopolamine treatment significantly ($P < 0.05$) increased acetylcholinesterase activity in brain as compared to control. However, piracetam and *Solanum melongena* (200 and 400 mg/kg, p.o) treatment significantly ($P < 0.05$) decreased acetylcholinesterase activity as compared to scopolamine treated group (tab. 4, fig. 4).

Oxidative stress parameters

The antioxidant activity of the enzymes such as superoxide dismutase (SOD) and catalase, were significantly inhibited in scopolamine treated group when compared with normal control group ($P < 0.05$). Piracetam and *Solanum melongena* significantly ($P < 0.05$) increased the activity of these antioxidant enzymes when compared to scopolamine treated group.

Scopolamine treatment significantly ($P < 0.05$) increased the brain MDA levels compared to control group. Piracetam and *Solanum melongena* significantly ($P < 0.05$) decreased brain MDA levels compared to scopolamine treated group (tab. 5, fig. 5, 6, 7).

The results illustrated in figure 5, 6 and 7 were presented as the mean ± SEM. Statistical analysis was done by ANOVA followed by Bonferroni's test. $P < 0.05$ was considered as statistically significant. Values are presented as mean ± SEM, n = 6.

Table 4. Effect of *Solanum melongena* extract on acetylcholinesterase activity in scopolamine treated rats.

Treatment groups (n = 6)	Acetylcholinesterase activity in μmol/min/mg protein
1. Vehicle control	83.57 ± 0.18
2. Positive control	158.1 ± 0.14*
3. Standard	98.13 ± 0.16#
4. Test 1	125.8 ± 0.2136#
5. Test 2	108.6 ± 0.2182#

*P < 0.05 compared with normal control group. #p<0.05 compared with scopolamine treated group. The results were presented as the mean ± SEM. Statistical analysis was done by ANOVA followed by Bonferroni's test. P < 0.05 was considered as statistically significant. Values are presented as mean ± SEM, n = 6.

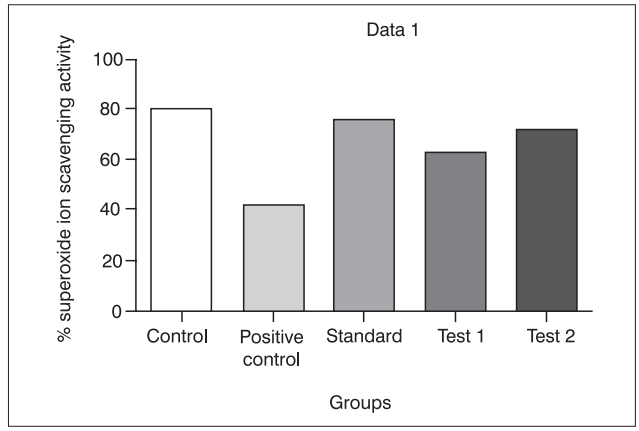

Fig. 5. Effect of *Solanum melongena* extract on percent of superoxide ion scavenging activity in scopolamine treated rat brain.

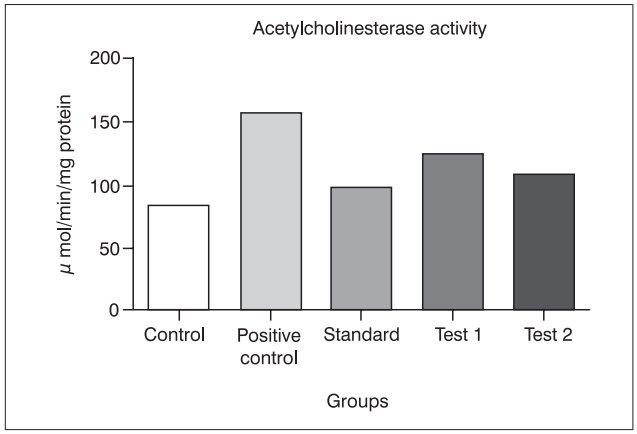

Fig. 4. Effect of *Solanum melongena* extract on acetylcholinesterase activity in scopolamine treated rats.

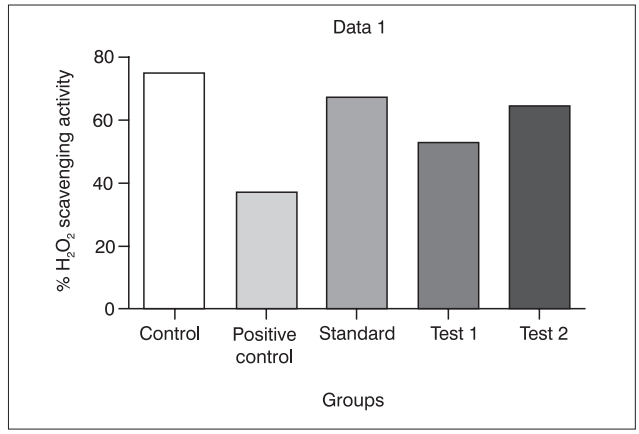

Fig. 6. Effect of *Solanum melongena* extract on percent of H_2O_2 scavenging activity in scopolamine treated rat brain.

Table 5. Effect of *Solanum melongena* extract on scopolamine induced oxidative stress parameters in rat brain.

Treatment groups (n = 6)	Percent superoxide ion scavenging activity	Percent H_2O_2 scavenging activity	LPO (nmol/mg tissue)
1. Vehicle control	80.50 ± 0.51	74.88 ± 0.26	25.55 ± 1.71
2. Positive control	42.32 ± 0.35*	36.78 ± 0.23*	46.32 ± 1.99*
3. Standard	75.22 ± 0.33#	67.47 ± 0.45#	28.90 ± 1.33#
4. Test1	63.28 ± 0.26#	53.10 ± 0.37#	37.89 ± 1.00#
5. Test2	71.72 ± 0.25#	64.22 ± 0.37#	33.62 ± 1.93#

*P < 0.05 compared with normal control group. #p<0.05 compared with scopolamine treated group. The results were presented as the mean ± SEM. Statistical analysis was done by ANOVA followed by Bonferroni's test. P < 0.05 was considered as statistically significant. Values are presented as mean ± SEM, n = 6.

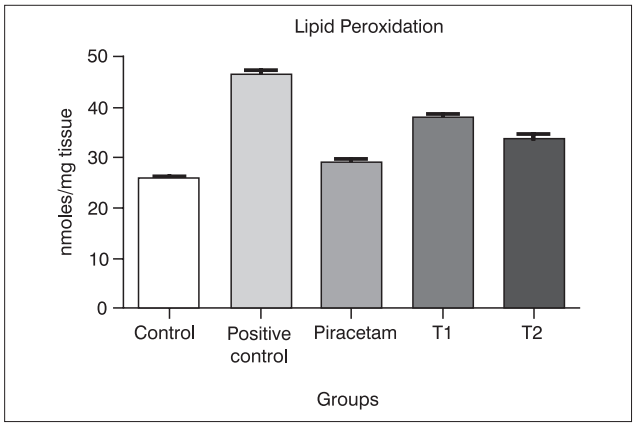

Fig. 7. Effect of *Solanum melongena* extract on lipid peroxidation in scopolamine treated rat brain.

Discussion

Memory is the process by which experiences are recorded and can be used to adapt their responses to the environment and it is vital for survival. Central cholinergic system is considered as the most important neurotransmitter involved in regulation of cognitive functions. The dementing condition that has received the most attention in the past decade is Alzheimer disease (AD). Impaired cognitive functions are the major features of AD.

Loss of cholinergic neurons in nucleus basalis magnocellularis of cortex is one of the most prominent features of AD, primarily accounting for memory loss. Scopolamine is a centrally acting cholinergic agent which causes impairment in learning. Treatment with drugs which increase cholinergic neurotransmission causes an improvement in cognitive deficits in AD (12).

Herbal medicine emphasizes prevention of disease, rejuvenation of our body systems and it extends the life span and makeslife healthy. Plant extracts may also provide a source of new compound as many synthetic drugs have been originated from herbal sources. *Solanum* species (eggplants) belong to the family of *Solanaceae* and the plant genus *Solanum* with over 1.000 species worldwide.

Eggplants have indigenous medicinal uses, which range from weight reduction to treatment of several ailments including asthma, skin infections and constipation. Various plant parts are used in decoction for curing ailments such as diabetes, leprosy, gonorrhea, cholera, bronchitis, dysuria, dysentery, asthenia and hemorrhoids. The present investigation is carried out on *Solanum melongena* L. (eggplant) for presence of its antiamnesic activity due to the presence of various phytoconstituents mainly flavonoids, polyphenols which might be responsible for exhibiting anti amnesic activity. The plant content (chlorogenic acid) was proved to possess central nervous system activity so it was chosen to evaluate antiamnesic activity (13).

Present study was evaluated on radial arm maze model and active avoidance paradigm to determine its efficacy in treating memory impairments induced by scopolamine. The anticholinesterase and antioxidant effect of *Solanum melongena* extract were being evaluated due to presence of chemical constituents like chlorogenic acid, nasunin, caffeic acid in it. The results suggest that *Solanum melongena* extract (200 and 400 mg/kg) has a considerable and significant effect in reducing cognitive impairments in rats. Many clinical studies have reported strong evidence that oxidative stress is involved in the pathogenesis of AD (14). Pretreatment with *Solanum melongena* extract (200 and 400 mg/kg, p.o.) produced a significant decrease in TBARS, SOD and catalase activities are restored.

Acetylcholinesterase is the enzyme responsible for acetylcholine hydrolysis which terminates the cholinergic transmission. The anticholinesterase activity was evaluated and extract was found to inhibit the acetylcholinesterase enzyme. Radial arm maze (RAM) performance is an appetitive motivated task and is also useful to assess spatial working memory and reference memory performance (15). Results of this study showed that oral administration of extract have decreased the occurance of working and reference memory errors significantly when compared with control group. In the active avoidance paradigm, avoidance responses were recorded it is clearly seen that there was general decrease in the performance in the active avoidance in scopolamine treated groups.

Conclusions

The present study demonstrates that beneficial effect of *Solanum melongena* L. (eggplant) on scopolamine induced amnesia. The extract significantly ameliorated the cognitive deficit. It showed significant antiamnesic activity as assessed by behavioral test using RAM and jumping box. Based on the results of behavioral and biochemical studies, hypothesize that eggplant extract may act directly as a free radical scavenger or regulator to inhibit acetylcholinesterase due to the presence of phytoconstituents mainly flavonoids, polyphenols which might be responsible for exhibiting antiamnesic activity. The results suggest that pretreatment with extract of *Solanum melongena* L. (eggplant) possess antiamnesic activity. Further studies are necessary to understand the mechanisms underlying the pharmacological activity of the constituents of the extract.

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